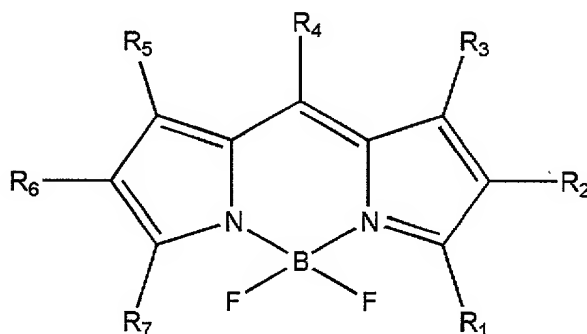


What is claimed is

1. A method of labeling poly(amino acids) comprising the steps of:

- 5 a. separating poly(amino acids) by gel electrophoresis, resulting in separated poly (amino acids);
- b. transferring said separated poly(amino acids) to a solid support, resulting in immobilized poly(amino acids) ;
- 10 c. combining said immobilized poly(amino acids) on said solid support with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl [carboxylic acid, sulfonic acid, or halogen], aryl, arylothenyl, arylbutadienyl, and heteroaryl [C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl];

provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen

atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

d. incubating the immobilized poly(amino acids) in the labeling mixture for a sufficient time for the dyes to form a covalent bond with said poly(amino acids), resulting in labeled poly(amino acids).

2. A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, Rx is a succinimidyl ester of a carboxylic acid.

3. A method, as claimed in Claim 1, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

4. A method, as claimed in Claim 3, wherein said solid support is made of materials that are poly(vinylidene difluoride) .

5. A method, as claimed in Claim 1, wherein said poly(amino acids) immobilized on said solid support has a molecular weight of 500 to-200,000 Daltons.

6. A method, as claimed in Claim 1, wherein said dye is present in the labeling mixture at a concentration of 0.10 micromolar to 10 micromolar.

7. A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-,

-(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-;

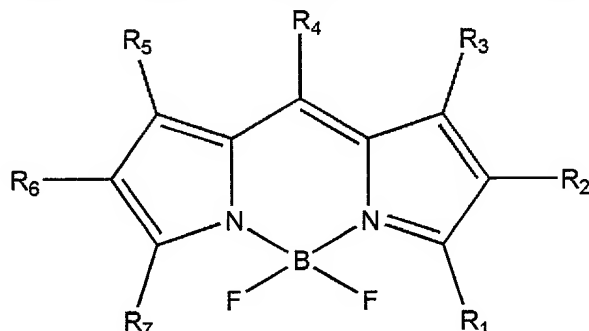
and Rx is a succinimidyl ester of a carboxylic acid.

8. A method, as claimed in Claim 7, further comprising adding a specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

9. A method of labeling poly(amino acids) bound to aptamers comprising the steps of:  
a. incubating immobilized aptamers with poly(amino acids) for a sufficient time to allow said poly(amino acids) to bind to their specific aptamers, resulting in immobilized poly(amino acids);

b. removing unbound poly(amino acids) that are not immobilized,

c. combining said immobilized poly(amino acids) with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, arylothenyl, arylbutadienyl, and heteroaryl;

provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,

two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and

only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen

atoms selected from the group consisting of C, N, O, P, and S and is composed of

any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

d. incubating the immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids), resulting in labeled poly(amino acids) that are bound to the aptamers.

10. A method, as claimed in Claim 9, wherein the dipyrrometheneboron difluoride dye's chemically reactive group is a succinimidyl ester of a carboxylic acid.

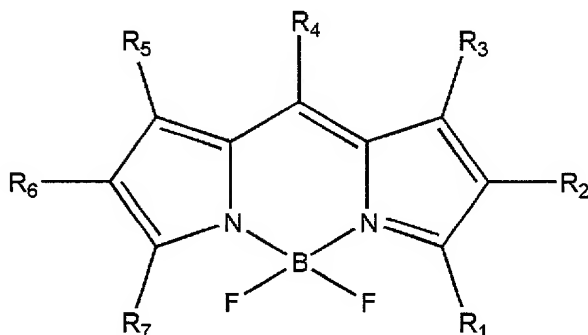
11. A method, as claimed in Claim 9, wherein said dipyrrometheneboron difluoride dye is present in the combined labeling mixture at a concentration of 0.10 micromolar to 10 micromolar.

12. A method, as claimed in Claim 9, wherein for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-; and Rx is a succinimidyl ester of a carboxylic acid.

13. A method, as claimed in Claim 12, further comprising adding a specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

14. A method of labeling immobilized poly(amino acids) in an array comprising the steps of:

a. combining an array of immobilized poly(amino acids) with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula



wherein each of  $R^1$  through  $R^7$  are independently selected from the group consisting of H, halogen,  $L-R_x$ , and substituted or unsubstituted  $C_1$ - $C_6$  alkyl, aryl, arylolethynyl, arylbutadienyl, and heteroaryl;

provided that one or more of  $R^1$  through  $R^7$  is H,

two or more of  $R^1$  through  $R^7$  is nonhydrogen, and

only one of  $R^1$  through  $R^7$  is  $-L-R_x$ , where L is a spacer having 1-24 nonhydrogen

atoms selected from the group consisting of C, N, O, P, and S and is composed of

any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-

nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur

bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and  $R_x$  is a

reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;

such that the dipyrrometheneboron difluoride dye has an absorption maximum

between 495 nm and 640 nm;

b. incubating said array with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids), resulting in the array of poly(amino acids) being labeled.

15. A method, as claimed in Claim 14, wherein for the dipyrrometheneboron difluoride dye, Rx is a succinimidyl ester of a carboxylic acid.

16. A method, as claimed in Claim 14, wherein said dipyrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0.10 micromolar - 10 micromolar.

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17. A method, as claimed in Claim 14, wherein for the dipyrrometheneboron difluoride dyes, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl;

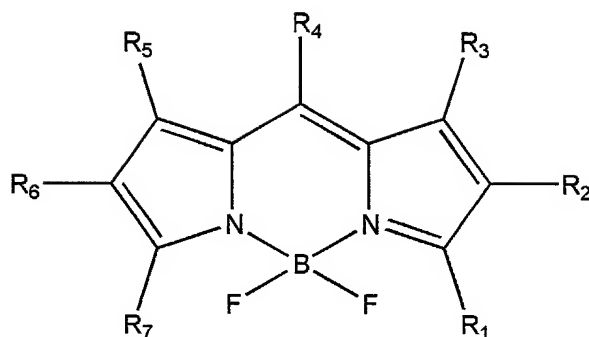
10 where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-,  
(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-;  
and Rx is a succinimidyl ester of a carboxylic acid.

15 18. A method, as claimed in Claim 17, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

20 19. A method, as claimed in Claim 14, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

20. A method of detecting poly(amino acids) comprising the steps of:

25 a. combining poly(amino acids) immobilized on a solid support; with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula



wherein each of  $R^1$  through  $R^7$  are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted  $C_1$ - $C_6$  alkyl, aryl, arylolethynyl, arylbutadienyl, and heteroaryl;

provided that one or more of  $R^1$  through  $R^7$  is H,

two or more of  $R^1$  through  $R^7$  is nonhydrogen, and

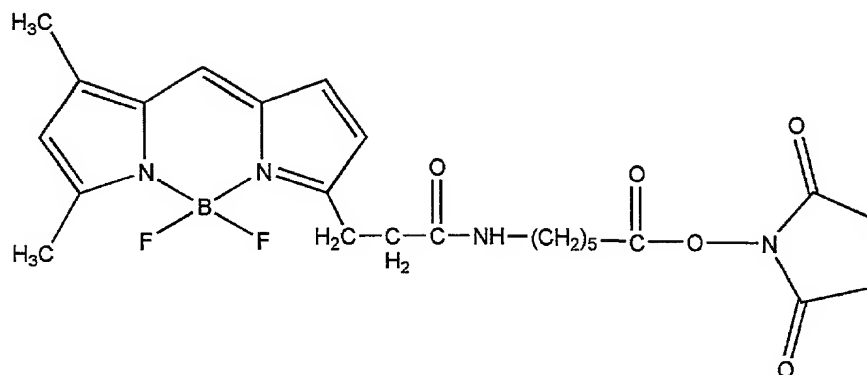
only one of  $R^1$  through  $R^7$  is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

b. incubating said immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids) resulting in labeled poly(amino acids);

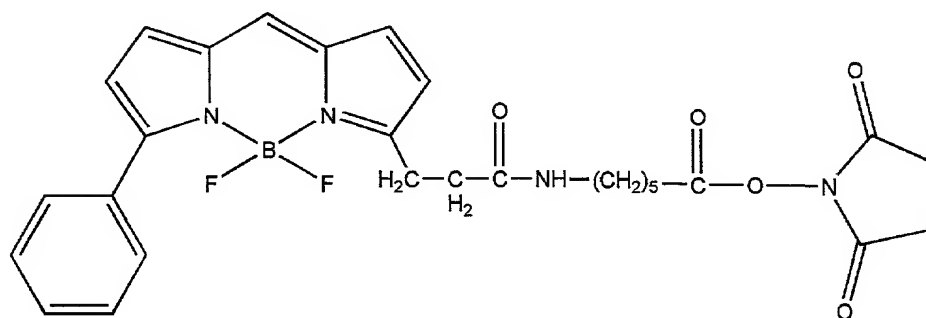
c. removing unbound dipyrrometheneboron difluoride dyes ;

d. illuminating said labeled poly(amino acids) to yield a fluorescent optical response to detect the corresponding labeled poly(amino acids).

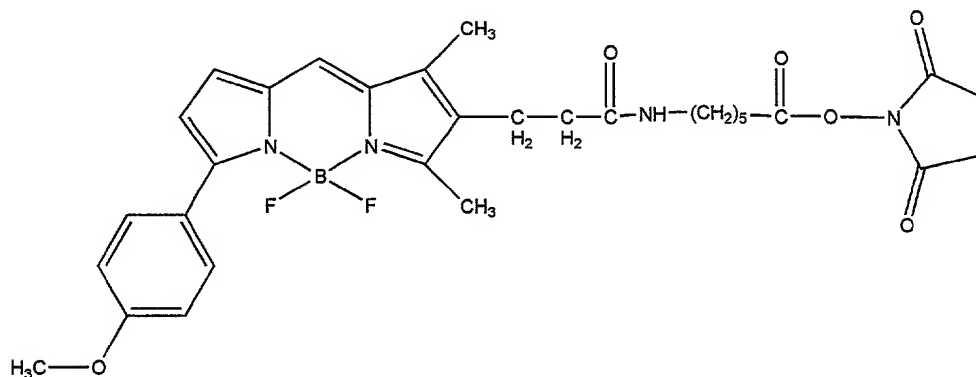
21. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



22. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

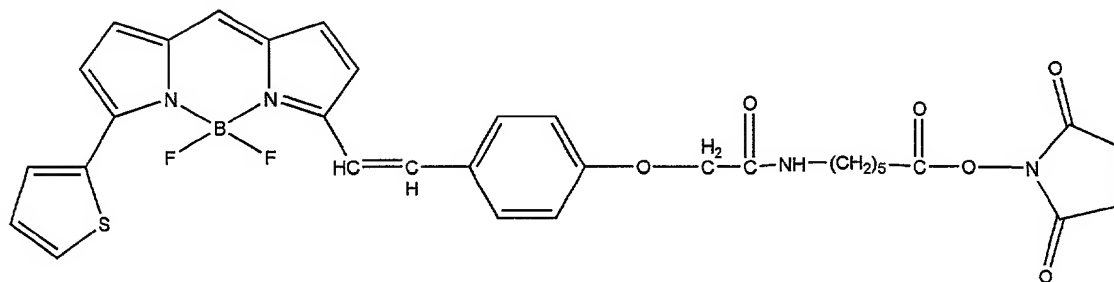


23. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



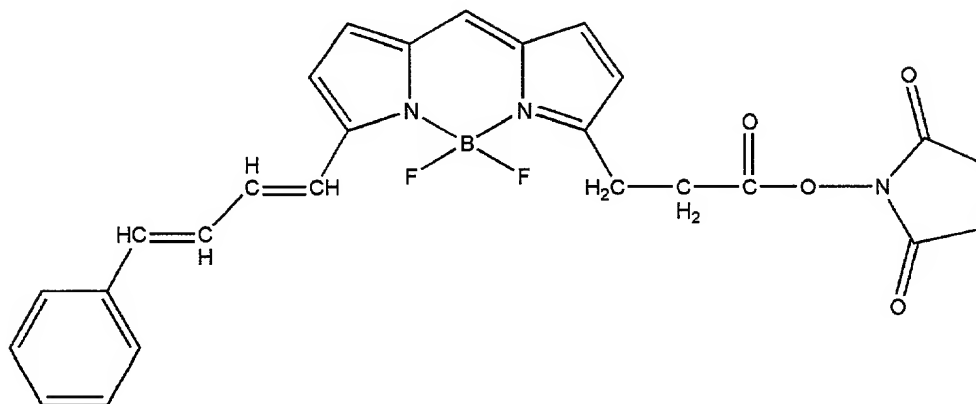


24. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



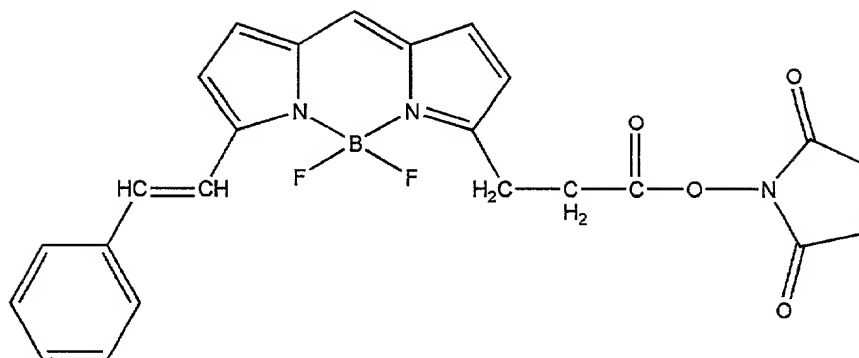
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25. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



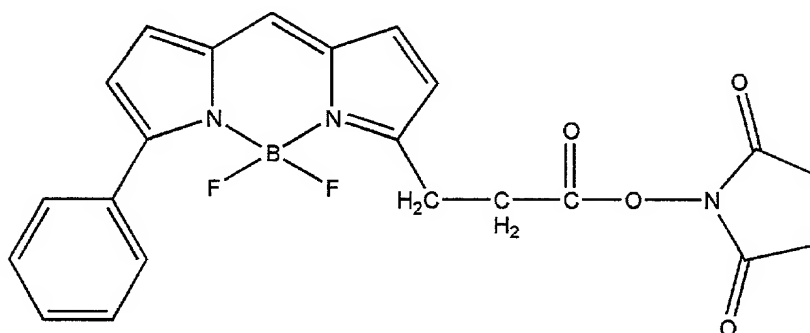
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26. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



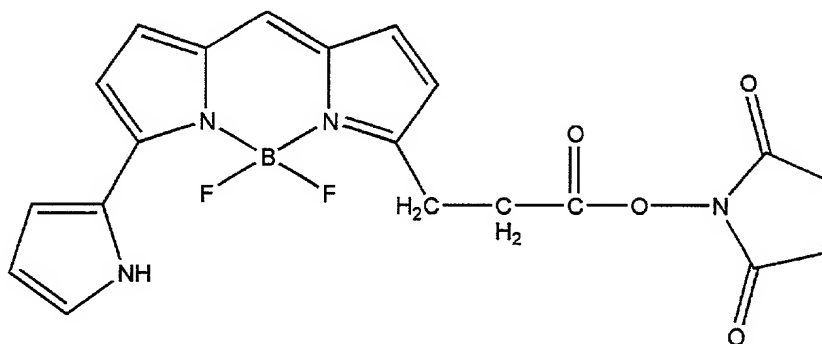
27. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

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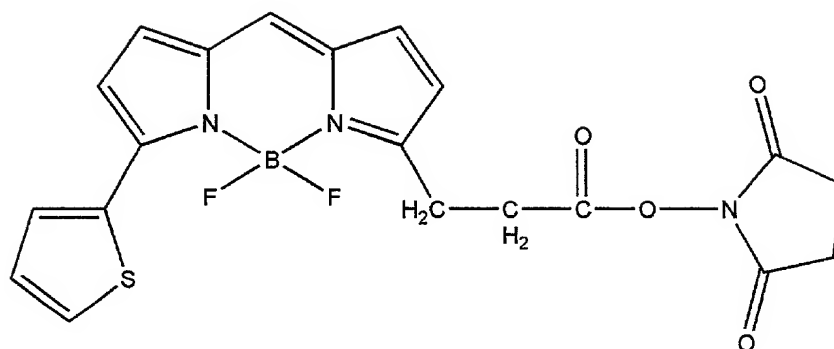


28. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

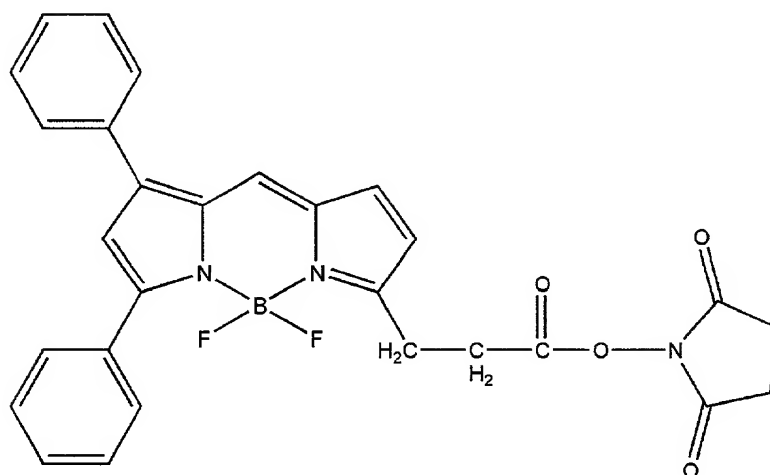
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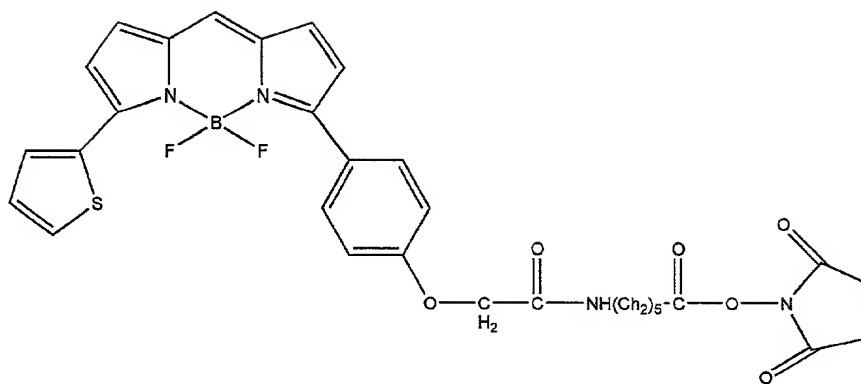
29. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



30. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



31. A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



32. A method, as claimed in Claim 20, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

33. A method, as claimed in Claim 32, wherein said solid support is made of materials that are poly(vinylidene difluoride).

34. A method, as claimed in Claim 20, wherein said poly(amino acids) on said solid support each have a molecular weight of between 500 Daltons and 200,000 Daltons.

35. A method, as claimed in Claim 20, wherein for said dipyrrometheneboron difluoride dye  $R^1$  is methyl or  $-L-Rx$ ;  $R^2$  is H, bromine, or  $-L-Rx$ ;  $R^3$  is H or methyl;  $R^4$  is H or  $-L-Rx$ ;  $R^5$  is H, methyl, or phenyl;  $R^6$  is H or bromine; and  $R^7$  is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where  $-L-$  is  $-(CH_2)_2-$ ,  $-(CH_2)_4-$ ,  $-OCH_2C(O)NH(CH_2)_5-$ ,  $-(CH_2)_2-C(O)NH(CH_2)_5-$ ,  $-(CH_2)_2C_6H_4OCH_2C(O)NH(CH_2)_5-$ ; and  $Rx$  is a succinimidyl ester of a carboxylic acid..

36. A method, as claimed in Claim 35, wherein said dipyrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0.10 micromolar to 10 micromolar, and

wherein said labeled poly(amino acids) are illuminated for five seconds or less.

37. A method, as claimed in Claim 20, further comprising adding a specific binding pair member that selectively binds to a target within said immobilized poly(amino acids) that is its complementary binding pair.

5 38. A method, as claimed in Claim 37, where said specific binding pair member contains a label that is an enzyme or a hapten.

39. A method, as claimed in Claim 37, where said specific binding pair member contains a label that is a fluorophore.

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40. A method, as claimed in Claim 37, further comprising:  
adding a secondary complementary binding pair member that contains a label and that selectively binds to the specific binding pair member.

15 41. A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is an enzyme.

42. A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is a fluorescent dye.

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43. A method, as claimed in Claim 41, wherein said enzyme is a peroxidase or a phosphatase.

44. A method, as claimed in Claim 43, wherein said peroxidase is horseradish peroxidase.

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45. A method, as claimed in Claim 43 wherein said phosphatase is alkaline phosphatase.

46. A method, as claimed in Claim 41, wherein said enzyme is capable utilizing a fluorogenic substrate to generate a detectable optical response.

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47. A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a fluorescent tyramide.

48. A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is a quinazolinone phosphate.

49. A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate.

50. A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a polyfluorinated xanthene.

51. A method, as claimed in Claim 40, wherein said secondary complementary binding pair is an antibody or an antibody fragment.

52. A method, as claimed in Claim 39, wherein said complementary specific binding pair member is a lectin.

53. A method, as claimed in Claim 39, wherein said specific binding pair member is biotin-binding protein that contains a label.

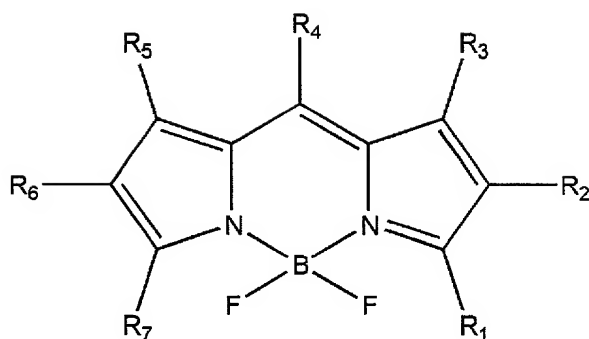
54. A method, as claimed in Claim 53, wherein said biotin-binding protein is streptavidin.

55. A method, as claimed in Claim 53, wherein said biotin-binding protein is NeutrAvidin.

56. A method, as claimed in Claim 37, wherein said specific binding pair member is an antibody or antibody fragment, an aptamer, a lectin, or a biotin-binding protein.

57. A kit for detection of poly(amino acids) immobilized on a solid surface, said kit comprising:

a. a dipyrrometheneboron difluoride dye of the formula:



wherein each of  $R^1$  through  $R^7$  are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted  $C_1$ - $C_6$  alkyl, aryl, arylothenyl, arylbutadienyl, and heteroaryl;

provided that one or more of  $R^1$  through  $R^7$  is H,  
two or more of  $R^1$  through  $R^7$  is nonhydrogen, and

only one of  $R^1$  through  $R^7$  is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

b. a specific binding pair member that contains a label and that selectively binds to a target that is its complementary binding pair.

58. A kit, as claimed in Claim 57, wherein the specific binding pair member contains a label that is an enzyme; wherein said enzyme is capable utilizing a fluorogenic substrate to generate a detectable optical response, said kit further comprising the fluorogenic substrate.

59. A kit, as claimed in Claim 57, wherein said specific binding pair member is an antibody or antibody fragment.

60. A kit, as claimed in Claim 57, wherein the specific binding pair member contains a label that is a fluorescent dye.

5 61. A kit, as claimed in Claim 57, wherein said specific binding pair member is a biotin-binding protein.

62. A kit, as claimed in Claim 61, wherein said biotin-binding protein is avidin, Neutravidin or streptavidin.

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63. A kit, as claimed in Claim 58, wherein said label is an enzyme that is a peroxidase or a phosphatase.

64. A kit, as claimed in Claim 63, wherein said peroxidase is horseradish peroxidase.

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65. A kit, as claimed in Claim 64, wherein said fluorogenic substrate peroxidase substrate that is a fluorescent tyramide.

66. A kit, as claimed in Claim 63, wherein said phosphatase is alkaline phosphatase.

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67. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is a 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate.

68. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase  
25 substrate that is a 2-(5'-chloro- 2'-phosphoryloxyphenyl)-6-chloro- 4(3H)-quinazolinone.

69. A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is ELF 39 reagent.

30 70. A kit, as claimed in Claim 58, wherein



for the dipyrrometheneboron difluoride dye,  $R^1$  is methyl or  $-L-Rx$ ;  $R^2$  is H, bromine, or  $-L-Rx$ ;  $R^3$  is H or methyl;  $R^4$  is H or  $-L-Rx$ ;  $R^5$  is H, methyl, or phenyl;  $R^6$  is H or bromine; and  $R^7$  is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl; where  $-L-$  is  $-(CH_2)_2-$ ,  $-(CH_2)_4-$ ,  $-OCH_2C(O)NH(CH_2)_5-$ ,  $-(CH_2)_2-C(O)NH(CH_2)_5-$ ,

5  $-(CH)_2C_6H_4OCH_2C(O)NH(CH_2)_5-$ ;

and  $Rx$  is a succinimidyl ester of a carboxylic acid;

the specific binding pair member is an antibody or a streptavidin that contains a label that is an alkaline phosphatase and the fluorogenic substrate is a 9H-(1,3-dichloro-9,9-

10 dimethylacridin-2-one-7-yl) phosphate, a 2-(5'-chloro- 2'-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone, or ELF 39 reagent.